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(54) Contrast agent for NMR imaging

(57) The agent has improved stability and results in an enhanced water proton relaxation rate. It comprises liposomes which contain paramagnetic ions bound to physiologically acceptable macromolecules.

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SPECIFICATION

Contrast agent for NMR imaging

The invention relates to novel contrast media for NMR-Medical Imaging. Amongst others the novel contrast media have an improved stability compared with preparations of similar properties; they result in enhanced water proton relaxation rate. The novel contrast media are provided in the form of liposomes containing paramagnetic ions bound to physiologically acceptable macro-molecules.

NMR imaging (MRI) is a comparatively new technique which provides a 3-dimensional picture of the human body or of certain organs thereof in a non-invasive manner. The diagonistic value of ^1H MRI is greatly enhanced when the proton density information is superimposed on proton relaxation time information. It is established that the proton relaxation times of tissue water reflect not only the composition, and the structural complexity of the tissue, but also is physiological or pathologic state MRI contrast agents are very useful for improving the delineation of structures of organs, for characterizing physiological functions and for the further differentiation of tissues.

For this purpose there are generally used paramagnetic ions or stable free radicals which dramatically shorten water relaxation times at relatively low concentrations. The use of such materials as contrast enhancing agents has two quite serious problems, namely the toxicity of the agents and the problem of delivery to the desired target tissues. Some of the most effective paramagnetic relaxation probes, such as Mn^{2+} and Gd^{3+} or stable nitroxides are quite toxic, even at low dosages. Furthermore the metabolic routes of these have not been fully established. The toxicity problem can be overcome to a certain extent by the complexing of such ions with a strong complexing agent, such as DTPA, EDTA, but this limits the use of the complexed agent to the blood stream and to blood vessels. Recently the use of the Mn^{2+}-DTPA entrapped in multilamellar liposomes was investigated by Caride et al., Mag.Resonance Imaging 2, 107(1984). It was found that the entrapment in liposomes alters the biodistribution of the metal chelate and that ^{54}Mn accumulation did very markedly increase in the spleen and in the liver, with some reduction in the heart and kidneys relative to free Mn-DTPA. The accumulation in the liver seems to indicate leakage of the complex from the liposomes and their subsequent dissociation.

There are provided contrast agents for NMR imaging in medicine. The MRI contrast enhancers of the present invention comprise paramagnetic ions bound to physiologically acceptable macromolecules which are entrapped within liposomes. The binding of the paramagnetic ions to macromolecules enhances the water proton relaxation rate and thus smaller quantities of such ions can be used. This is of importance in view of the substantial toxicity of such ions. The macromolecule-bound ions tend to leak to a much lesser degree from the liposomes, thus resulting in an extended useful lifetime inside the body. The contrast agents of the invention, due to the use of specific liposomes, make possible an improved targeting to specific organs as well as to normal or tumorous tissues. Liposome types developed for targeting drugs to certain organs of the human body can be used for this effect, see for example, Weinstein, UCLA Symp.Mol.Cell Biol. *4*, 441 (1983). The paramagnetic ions may be bound to suitable macromolecules. Macromolecules of choice are certain proteins, and especially human serum proteins so as to reduce immune reaction problems. The binding properties of the protains can be used for the bonding of the ions: BSA is known to bind manganese and gandolinium with proton relaxation enhancement: Biochem *2*, 910 (1963) and Biochem *10* (1971), 2834. Experiments carried out by us have shown that there can be advantageously used human serum albumin as well as beta- and gamma-globulins. The experiments have demonstrated that a 10% (w/w) solution of such protein dialyzed against 1 mM Mn^{2+}, the fraction of bound Mn^{2+} was 68%, 53% and 14% respectively for the above defined three types of serum proteins, respectively.

According to a further embodiment of the invention, the pramagnetic ions are complexed by means of a strong complexing agent such as DTPA or EDTA. Ions of choice are Mn^{2+} and Gd^{3+}, but the same system can be used with other suitable metal ions. The thus obtained complexes give a significant relaxation enchancement, and the entrapment of such complex inside the liposomes does not reduce the relaxation effect which seems to be due to the fast diffusion of water molecules across the liposome membrane system, thus producing a fast exchange on the NMR time scale and thus a weighed average of relaxation times.

The preparation of liposomes entrapping proteins is well known in the art and need not be described here in detail. See, for example, textbooks such as Liposome Technology, Vol. 1 to 3, Boca Baton, Florida, CRC Press, 1984.

In the following Example the vesicles were prepared as set out on Biochemistry *20* 833 (1981).

The following Examples are provided in order to illustrate the present invention and they are to be construed in a non-limitative manner. It is clear that a variety of different ions, proteins, chelating agents and mode of preparation of complexes and liposomes can be resorted to

without departing from the scope and spirit of the invention.

EXAMPLES

EXAMPLE 1:

The starting material was 0.3 ml egg lecitine (phosphatidyl choline, Sigma) in dioxane. The dioxane was removed by evaporation in a stream of nitrogen. 0.5 ml of $CHCl_3$ was added, then evaporated and lyophilized. 0.06 gr. n-octyl-β-D-glucopyranoside was added with 0.5 ml $CHCl_3$. The mixture was shaken, evaporated and lyophilized. 1 ml of 10% human serum albumin solution with 2 mM. $MnCl_2$, Hepes 20 mM, NaCl 130 mM, was added and the solution was dialyzed against two changes of 250 ml of the same solution without the protein's first dialysis for 24 h., and the second one—for 48 h. The content of the dialysis bag was washed by repeated (3 times) ultracentrifugation at 5°C, each time for 1 h. The final precipitate consists of washed vesicles, which contain Mn-HSA.

EXAMPLE 2:

A run was carried out as in Example 1, except that 10% β-Globulin was used instead of HSA. Vesicles were obtained in a similar manner.

EXAMPLE 3:

A run was carried out as in Example 1, except that 10% α-Globulin was used instead of HSA. Similar vesicles were obtained.

EXAMPLE 4:

A run was carried out as in Examples 1–3, but with 1 mM $MnCl_2$ instead of 2 mM. Vesicles containing a corresponding concentration of Mn^{2+} were obtained.

EXAMPLE 5:

Runs were carried out as in Examples 1 and 4, but with IgG-EDTA conjugate. Vesicles containing this conjugate with the Mn^{2+} were obtained.

EXAMPLE 6:

Runs were carried out as in Examples 1 and 4, but with HSA-EDTA conjugate. Vesicles containing the conjugate with Mn^{2+} were obtained.

EXAMPLE 7:

A number of runs were carried out as in Examples 1–6, but with Gd Cl_3 replacing $MnCl_2$. Vesicles containing the bound Gd^{3+} cations were obtained.

EXAMPLE 8:

Runs were carried out as in Examples 1, 4 and 7, except that IgG-DTPA conjugate replaced the HSA. Corresponding vesicles were obtained.

EXAMPLE 9:

Runs were carried out as in Examples 1, 4 and 7, except that HSA-DTPA conjugate replaced the HSA. Corresponding vesicles were obtained.

Results of Manganese Binding and Proton Relaxation Rates for Liposomes containing Mn^{2+} and Serum Proteins

In the following there is presented a series of examples of the effects observed:

There were measured by atomic absorption manganese ion concentrations in the buffers (blank) and in the suspensions of the liposomes, which contained 10% (w/w) of proteins from human serum. The volume, occupied by the liposomes, was about 20% of the suspension. The excess manganese concentration in the suspension over that of the buffer indicates binding of manganese to the proteins in the vesicles. It is seen from the Table that the largest binding was obtained for the serum albumine.

The measurements were made in two typical frequencies: 21 MHz and 42 MHz, which are used in NMR imaging.

The results of the T_1 relaxation time show a dramatic (up to 33-fold) decrease of T_1 over that of the blank, which contained manganese in equilibrium with the liposomes. Even when we normalise the results to manganese concentration, a relaxation enhancement of up to factor of 18 is obtained. The best results were obtained for albumin as it binds more Mn^{2+} and it gives also large relaxation enhancement.

Corresponding results were obtained with the liposomes containing Gd^{3+}.

The results for Mn^{2+} and Gd^{3+} bound to protein conjugated with EDTA and DTPA give less relaxation per metal ion, but more metal ions bound per protein. Therefore, the choice between

the different systems depends on the particular application and the clinical results.

The T_1 for suspensions of liposomes containing human serum albumin and Mn^{2+} ions at 21 and 42 MHz are given in Table 2. The concentrations of free manganese ions were kept constant throughout the preparation of the liposomes, including during the process of removal of external proteins. Thus, the additional Mn^{2+} concentrations in the liposome suspensions are due to Mn^{2+} binding to the proteins inside the liposomes.

For control experiments we measured T_1 relaxation times containing "empty vesicles" i.e. vesicles containing buffer without Mn^{2+}, as well as vesicles containing Mn^{2+} at the same concentration as the outside solutions. Although there was some shortening of T_1 in these samples compared with the blank solutions, the effect of vesicles containing HSA on T_1 relaxation rates is much larger. A comparison to solutions of serum albumin as described in Table 1 should take into consideration the small amount of albumin and bound Mn^{2+} in the suspension of the liposomes (Table 2). In fact, the normalized effect of the bound Mn^{2+}, $T_{1b}^{-1}/\Delta Mn^{2+}$ is similar in the two experiments. In an additional experiment which is not described in Table 2 we washed vesicles loaded with 10% HSA and 3mM Mn^{2+} with buffer solution without Mn^{2+}.

The results for the total Mn^{2+} concentration in the suspension as measured by atomic absorption were $[Mn^{2+}]=0.31$ mM and $T_1=46.3$ ms at a frequency of 42 MHz. The molar relaxivity, $T_1^{-1}/[Mn^{2+}]=69.7$ is comparable to the previous experiments. Thus, the fact that the bound manganese was enclosed in liposomes did not affect its relaxation enhancing properties.

It can be concluded that the relaxation obtained in the systems of the invention is greater by a large factor for the same amount of the toxic, paramagnetic metal ions.

Furthermore, toxicity is reduced significantly since the metal ions are entrapped in the liposomes.

TABLE 1

Sample	Mn^{2+}, mM	Frequency 21 MHz T_1, ms	T_{1p}^{-1}, s^{-1}	$T_{1p}^{-1}/\Delta Mn^{2+}$	Frequency 42 MHz T_1, ms	T_{1p}^{-1}, s^{-1}	$T_{1p}^{-1}/\Delta Mn^{2+}$
Blank		3000			3000		
Albumin	-	980	-		1020	-	-
β-Globulin	-	1020	-		1180	-	-
γ-Globulin	-	1210	-		1720	-	-
Blank	0.75	156	6.1	8.1	176	5.4	7.1
Albumin	2.34	4.7	206.	130.	6.1	158.	99.
β-Globulin	1.62	11.6	67.	77.	16.0	57.	65.
γ-Globulin	0.87	11.5	16.6	137	54.5	11.4	94
Blank	1.31	84.	11.6	9.8	93.	10.9	8.2
Albumin	3.12	4.2	226.	125.	5.0	169	104.
β-Globulin	2.65	7.4	123	92.	3.6	105	78.
γ-Globulin	1.51	25.5	27.3	136	30	22.2	111
Blank	2.64	44.	22.4	3.5	52.	18.9	7.2
Albumin	5.93	3.5	263.	80.	4.2	219.	66.
β-Globulin	4.99	4.7	190.	81.	5.1	173	76.
γ-Globulin	3.07	15.0	44.	102.	19.5	32.	74.

a) $T_{1p}^{-1} = T_1^{-1} - T_1(0)^{-1}$

where $T_1(0)$ is the value of T_1 of an identical solution without a paramagnetic ion.

TABLE 2

Water Proton Spin-Lattice Relaxation Times[a] in Suspensions of Vesicles with and without Human Serum Albumin and Mn^{2+}[b]

Blank		Empty vesicles[c]		Vesicles containing free Mn^{2+}[d]		Vesicles containing HSA and Mn^{2+}[e]			
$[Mn^{2+}]$ (mM)	T_1 (ms)	$[Mn^{2+}]$ (mM)	T_1 (ms)	$[Mn^{2+}]$ (mM)	T_1 (ms)	$[Mn^{2+}]$ (mM)	[HSA] (mM)	T_1[f] (ms)	$T_{1p}^{-1}/\Delta Mn^{2+}$[g] ($s^{-1}$ mM^{-1})
0.455	244	0.545	168	0.455	120	0.758	0.222	36	64.2
0.93	123	1.09	85	0.93	75	1.213	0.195	25	97.9
1.86	67	2.18[h]	46	1.86[i]	44	2.52	0.248	15	66.6

[a] At NMR frequency of 42 MHz.

[b] All solutions contained 130 mM NaCl, 20 mM Hepes buffer pH 7.0.

[c] Vesicles contained buffer as in footnote *b*. Mn^{2+} was added to the outside solution.

[d] Vesicles prepared by dialysis against solutions identical to those given as Blank.

[e] Vesicles prepared as described in the experimental solution. They were washed with the solutions given as Blank.

[f] T_1 relaxation times of the same solutions at a frequency of 21 MHz were 33, 25.5, and 14 ms, respectively.

[g] T_{1p}^{-1} is the difference between T_1^{-1} of the suspensions of vesicles with HSA and Mn^{2+} and those containing Mn^{2+} only. ΔMn^{2+} is the difference of Mn^{2+} concentration in the same two suspensions.

[h] Diameter of vesicles ± standard deviation: 340 ± 74 nm.

[i] Diameter of vesicles ± standard deviation: 402 ± 119 nm.

CLAIMS

1. An MRI contrast enhancer comprising a liposome containing macromolecule-bound paramagnetic ions.

2. An MRI contrast enhancer according to claim 1 where the paramagnetic ions are selected from Mn^{++} and Gd^{+3}.

3. An MRI contrast enhancer wherein the macromolecules are physiologically acceptable proteins.

4. An MRI contrast enhancer according to claims 3, wherein the protein is selected from serum protein.

5. An MRI contrast enhancer according to claim 4, where the serum protein is selected from serum albumin, beta-globulin and gamma globulin.

6. An MRI contrast enhancer according to any of claims 1 to 5, wherein the ions are bound to the protein by absorption forces of the protein.

7. An MRI contrast enhancer according to claims 1 to 5, wherein the paramagnetic ions are complexed with a strong complexing agent.

8. An MRI contrast enhancer according to claim 7, where the complexing agent is EDTA or DTPA.

9. An MRI contrast enhancer according to claims 1 to 8, where the liposome (vesicle) is a phospholipid liposome.

10. An MRI contrast enhancer according to claims 1 to 8, wherein there is used a synthetic polymer liposome.

11. MRI contrast enhancer systems for use as NMR medical imaging agents, substantially as hereinbefore described and with reference to any of the Examples.

12. An MRI contrast enhancer according to any of claims 1 to 11 in injectable unit dosage form.

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